1225899

THE UNITED STATES OF AMERICA

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UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

September 15, 2004

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APPLICATION NUMBER: *60/562,496*
FILING DATE: *April 14, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US04/25026*

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
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PTO/SB/16 (01-04)
Approved for use through 07/31/2006. OMB 0651-0032
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 389270013 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Dusan	Miljkovic	San Diego, CA
Jovan	Hranisavljevic	Belgrade, Yugoslavia
Zbigniew	Pietrzkowski	San Diego, CA

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Naturally Occurring and Synthetic Compounds That Modulate Glucose Metabolism

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

[X] Customer Number: 34284

OR

[ ] Firm or Individual Name					
Address					
Address					
City		State		Zip	
Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS *(check all that apply)*

[X] Specification *Number of Pages* 48
[ ] CD(s), Number _____
[ ] Drawing(s) *Number of Sheets* _____
[ ] Other (specify) _____
[ ] Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[X] Applicant claims small entity status. See 37 CFR 1.27.
[ ] A check or money order is enclosed to cover the filing fees.
[X] The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 502191
[ ] Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount ($): 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.
[ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

[Page 1 of 1]

Respectfully submitted,

SIGNATURE _____

Date 04/14/04

REGISTRATION NO. 46697 *(If appropriate)*

Docket Number: 100700.0035PRO

TYPED or PRINTED NAME Martin Fessenmaier

TELEPHONE 714-641-5100

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

[X] Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete if Known	
Application Number	
Filing Date	April 14, 2004
First Named Inventor	Dusan Miljkovic
Examiner Name	
Art Unit	
Attorney Docket No.	100700.0035PRO

METHOD OF PAYMENT *(check all that apply)*

[ ] Check [ ] Credit card [ ] Money Order [ ] Other [ ] None

[X] Deposit Account:

Deposit Account Number: 502191

Deposit Account Name: Rutan & Tucker

The Director is authorized to: *(check all that apply)*

[X] Charge fee(s) indicated below [X] Credit any overpayments

[X] Charge any additional fee(s) or any underpayment of fee(s)

[ ] Charge fee(s) indicated below, except for the filing fee

to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below		Fee Paid
Total Claims		-20** =		X	=
Independent Claims		- 3** =		X	=
Multiple Dependent					=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($)

SUBMITTED BY				(Complete *if applicable*)	
Name (Print/Type)	Martin Fessenmaier	Registration No. (Attorney/Agent)	46697	Telephone	714-641-5100
Signature				Date	April 14, 2004

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If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

NATURALLY OCCURRING AND SYNTHETIC COMPOUNDS THAT MODULATE GLUCOSE METABOLISM

This application makes specific reference to our co-pending provisional applications with the serial numbers 60/499,637 (filed 09/02/03), 60/493,447 (filed 08/08/03), and the provisional application entitled "Dietary Supplements for Metabolic Modulation", filed on 4/13/04, PCT applications with the serial numbers PCT/US01/07527 (filed 03/08/01), PCT/US02/07199 (filed 03/08/02), and U.S. Application with the serial number 10/668,921 (filed 09/23/03), all of which are incorporated by reference herein.

Detailed Description

The inventors contemplate compounds, compositions, and methods for prevention and/or treatment of various diseases that are associated with catabolism, utilization, and metabolism of energy carriers, and particularly with glucose catabolism, utilization, and metabolism. Various aspects of the inventive subject matter are described in the presentation materials below.

Furthermore, and with particular reference to the bioavailability studies shown below, the inventors recognize that various aspects of metabolism in a mammal may be influenced by one or more of contemplated compounds, which may even naturally occur (either via synthesis in the mammal or via dietary uptake) in such mammals. Therefore, the inventors contemplate that certain aspects of metabolic state in a mammal may be diagnosed by determination of one or more of the contemplated compounds. For example, by determination of at least of kinetin, zeatin, dihydrozeatin, and acetylguanine (or their corresponding ribosides), a predisposition or likelihood of developing type II diabetes, dyslipidemia, or syndrome X may be determined (*e.g.*, if these compounds are found in serum below a predetermined level). Similarly, onset, type, and/or presence of diabetes and other conditions may be confirmed using such methods. Of course, it should be recognized that the concentration may be determined from any body fluid using methods well known in the art, or indirectly via their metabolites or associated reactions (*e.g.*, cytokinin oxidase enzyme coupled test).

NATURALLY OCCURRING AND SYNTHETIC COMPOUNDS THAT MODULATE GLUCOSE METABOLISM

(Non-confidential version was presented at the 3rd International Symposium on AMP-activated protein kinase, held in Lorne Victoria, Australia, 23-26 March 2004)

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Presentation Outline

* **Early Investigations:**

  - ***Plant Extracts*** ***-PE1/PE2 (In Vitro; In Vivo)***

* **Characterization of Active Principles from PE1/PE2**

* **Experiments On Chemical Entities:**

  - ***In Vitro*** ***-Ex Vivo*** ***-In Vivo***

* ***MitoChroma Research Compounds: Next Steps***

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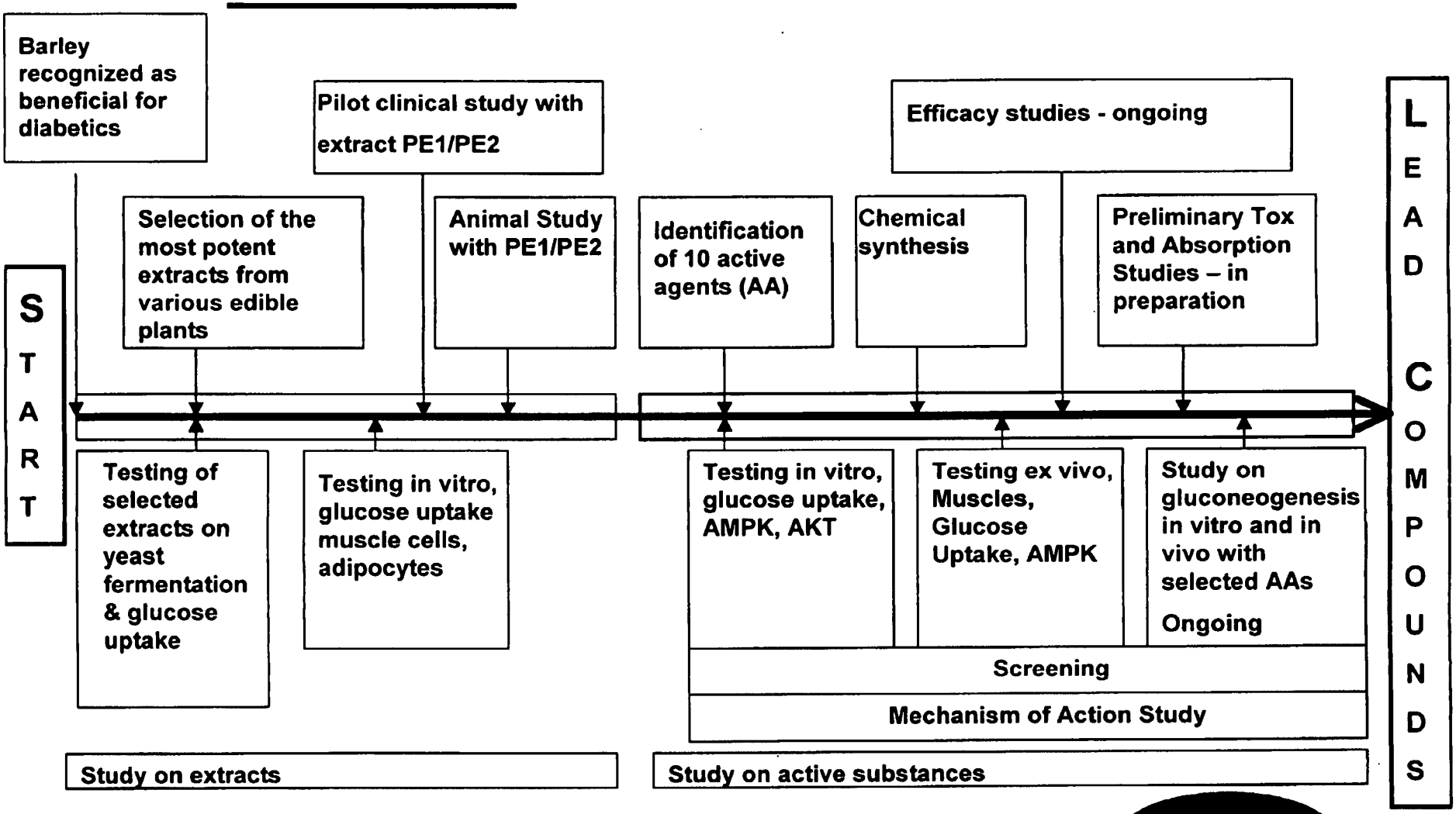
Timeline of the Project

Flow Chart



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Our Early Investigations

Discovery Phase Experiments
Yeast Fermentation Stimulation by Various Edible Plant Extracts

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NATURAL EXTRACTS FOUND TO STIMULATE YEAST FERMENTATION

1. **Two extracts** derived from edible plants, specifically prepared through selective extraction, comprised the starting materials for our studies.

2. These **extracts**, as well as later specific **fractions** of these (and other) extracts, (also obtained by our proprietary selective extraction process and/or preparative HPLC), were observed to be potent stimulators of fermentation of glucose in baker's yeast.

3. **Legend**: **P**lant **E**xtract **1** = **PE1**
Plant **E**xtract **2** = **PE2**

4. Overall potency of a combination of PE1 and PE2 was *synergistic* in regards to increase in yeast fermentation rates.

5. Activities revealed up to a four-fold increase in yeast fermentation.

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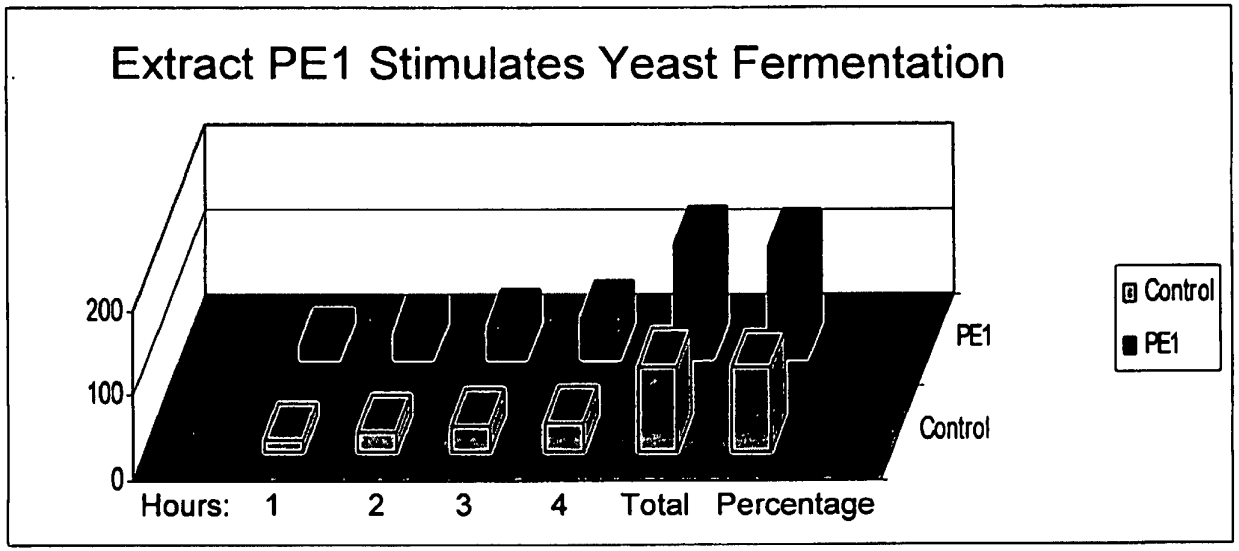
PLANT EXTRACTS AS YEAST FERMENTATION RATE ENHANCERS



A typical kinetics observed when yeast was treated with a crude edible plant extract. In the example above, the fermentation rate of PE1 was determined by measuring carbon dioxide evolution over 4 hours.

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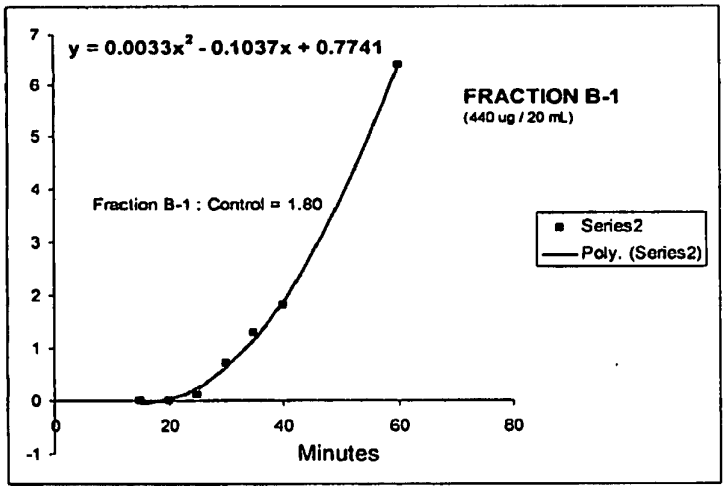
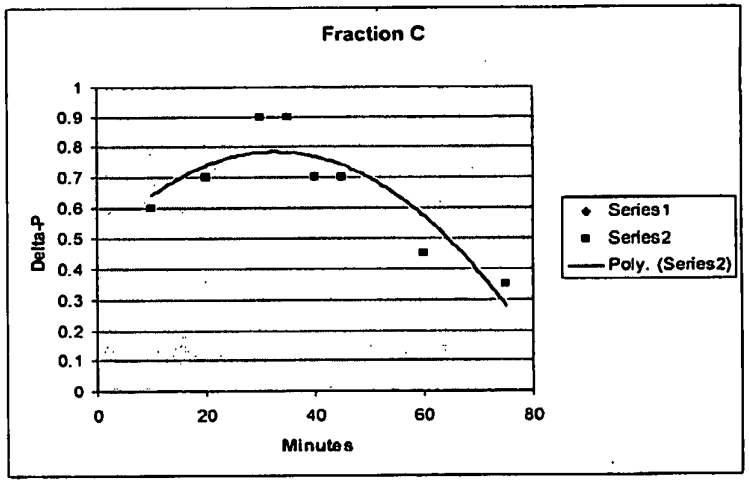
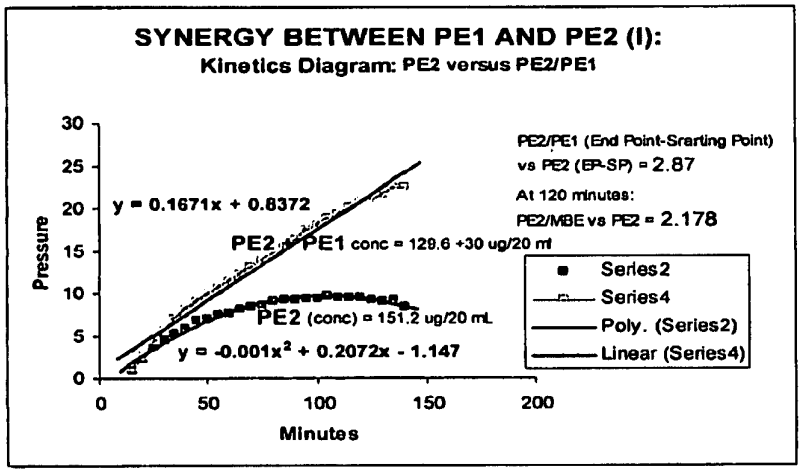
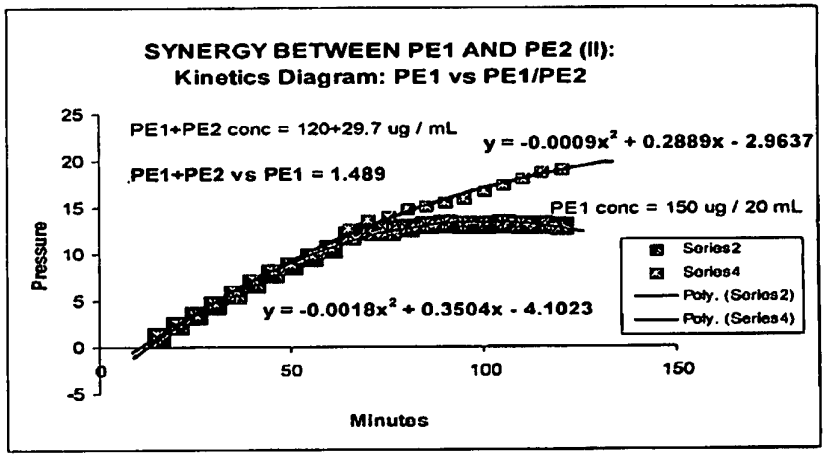
Separate Fractions Strongly Stimulate Yeast Fermentation Showing Different Kinetics





Synergy Exhibited by PE1 and PE2 in Combination





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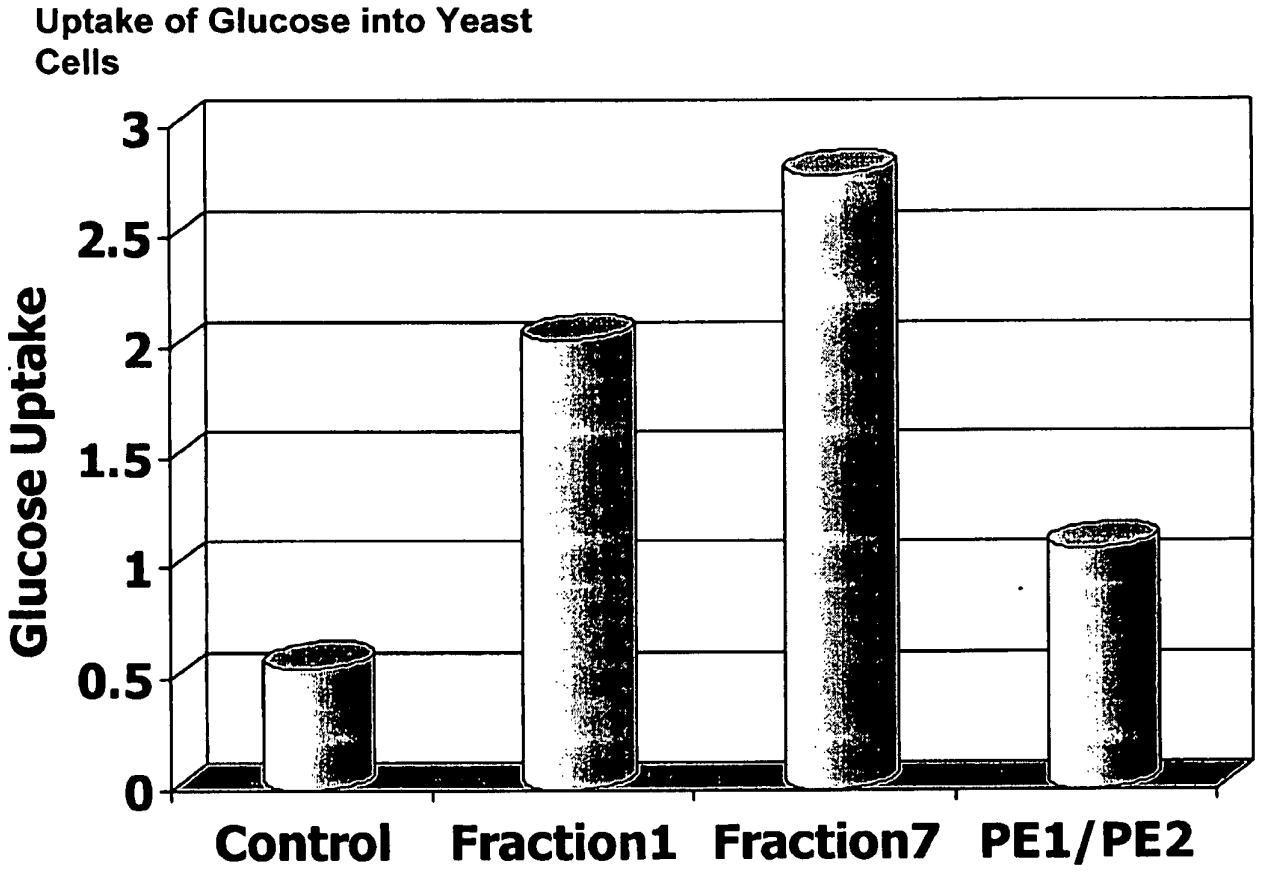
PLANT EXTRACTS AS GLUCOSE UPTAKE ENHANCERS FOR YEAST CELLS



Control expresses glucose uptake in presence of medium only

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In Vitro and *In Vivo* Experiments on PE1/PE2

Discovery Phase Experiments

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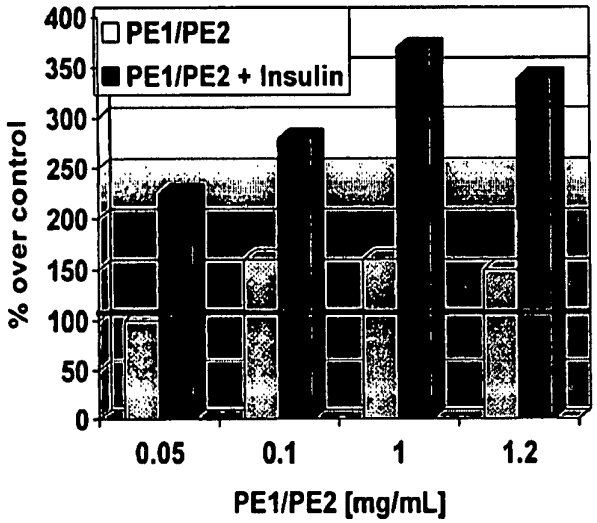
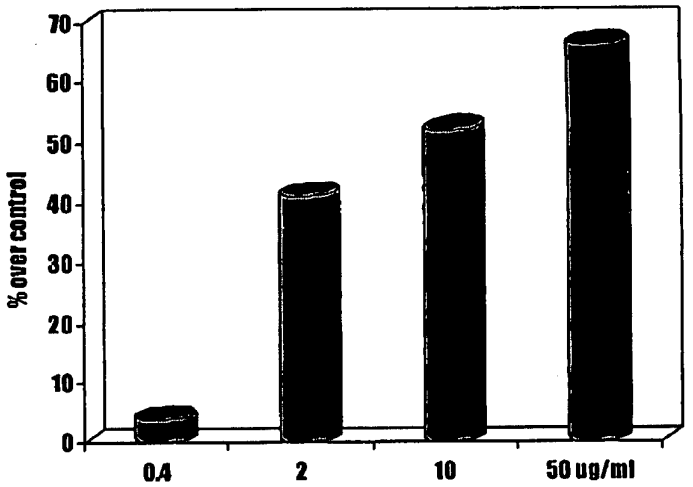
PE1/PE2 stimulates glucose uptake in rat adipocytes and L6 myoblasts *in vitro*.





•Uptake of 1-deoxy-D-[3H] glucose in primary culture of rat adipocytes was measured in presence of PE1/PE2 alone, insulin alone, and a combination of the two.

•Red line represents effect of 100nM of insulin under the experimental conditions.

PE1/PE2-Stimulated Dose-dependent Glucose Uptake into L6 Muscle Cells

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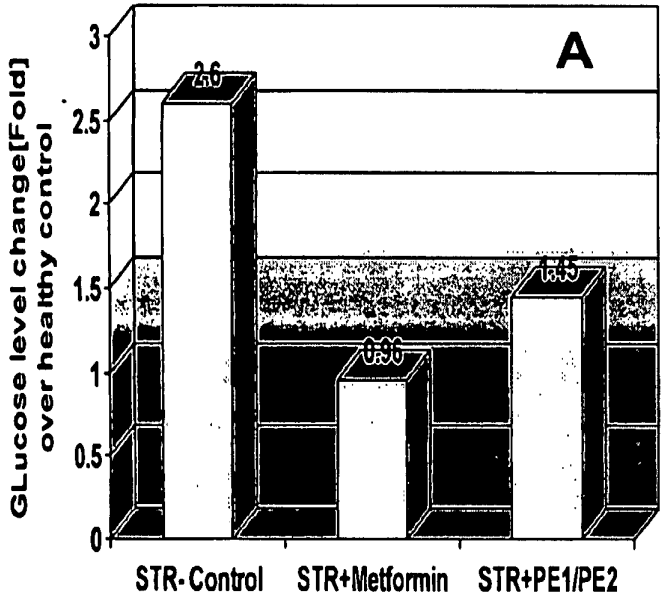
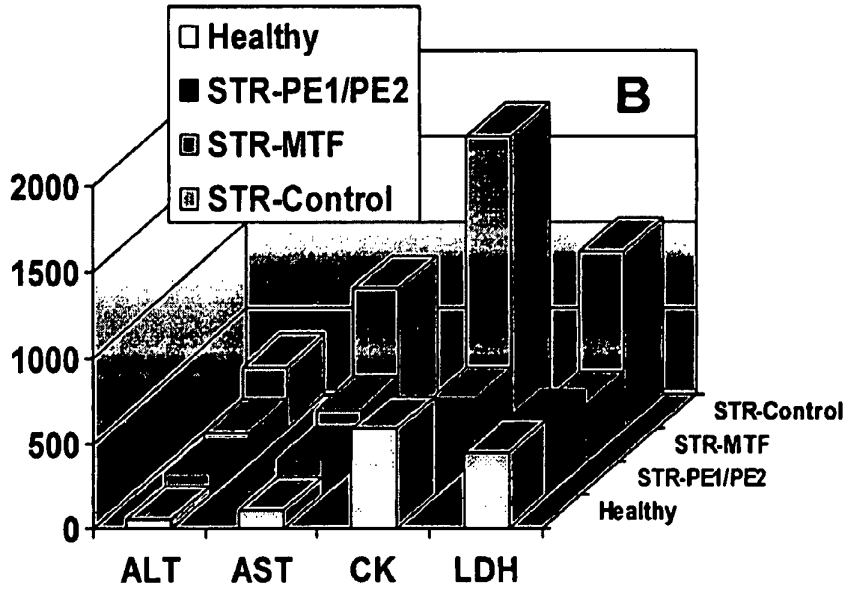
Effect of PE1/PE2 on Streptozocin rats

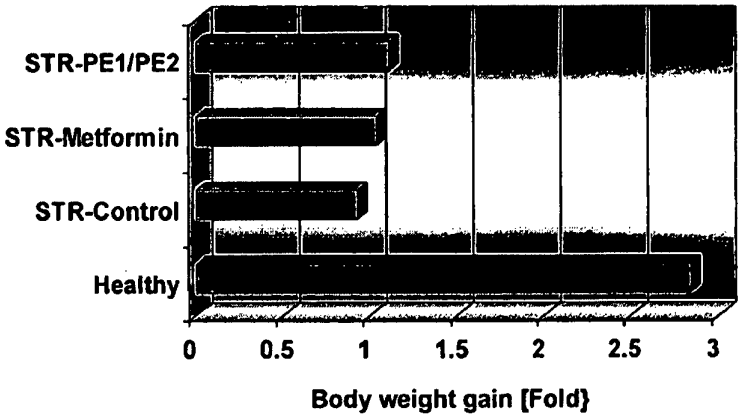
A: Changes in blood glucose levels



B: Changes of liver enzymes



C: Body weight changes



C

- PE1/PE2 or Metformin was delivered in drinking water
- Rats were treated for four weeks.

PE1/PE2: 85mg/kg
Metformin (MTF):500mg/kg

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Effect of PE1/PE2 on Streptozocin rats Observations

PE1/PE2 dosage: **85mg/kg**

Metformin (MTF) dosage: 500mg/kg

- **PE1/PE2 extract reduced blood glucose levels comparable to Metformin**
- **PE1/PE2 greatly improved liver enzymes over streptozocin group and equivalent to Metformin**
- **PE1/PE2 prevented body weight loss more effectively than Metformin**

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HUMAN PILOT CLINICAL STUDY ON "PE1/PE2"

1. A combination of Edible Plant Extracts (PE1/PE2), specifically prepared through selective extraction, was used in a human pilot study.

2. The study was done with 10 diabetes type 2 patients for ninety days. The total daily dose was 7.5 gr (3 x 2.5 gr) per patient administered orally. Selected blood analyses were performed at 0, 45 and 90 days.

3. Results revealed:
   a. 14% decrease in glucosylated hemoglobin
   b. 20% decrease in fasting and postprandial serum glucose
   c. 20% decrease in LDL/HDL ratio
   d. Significant improvement of glucose tolerance

Results illustrated in following four slides:

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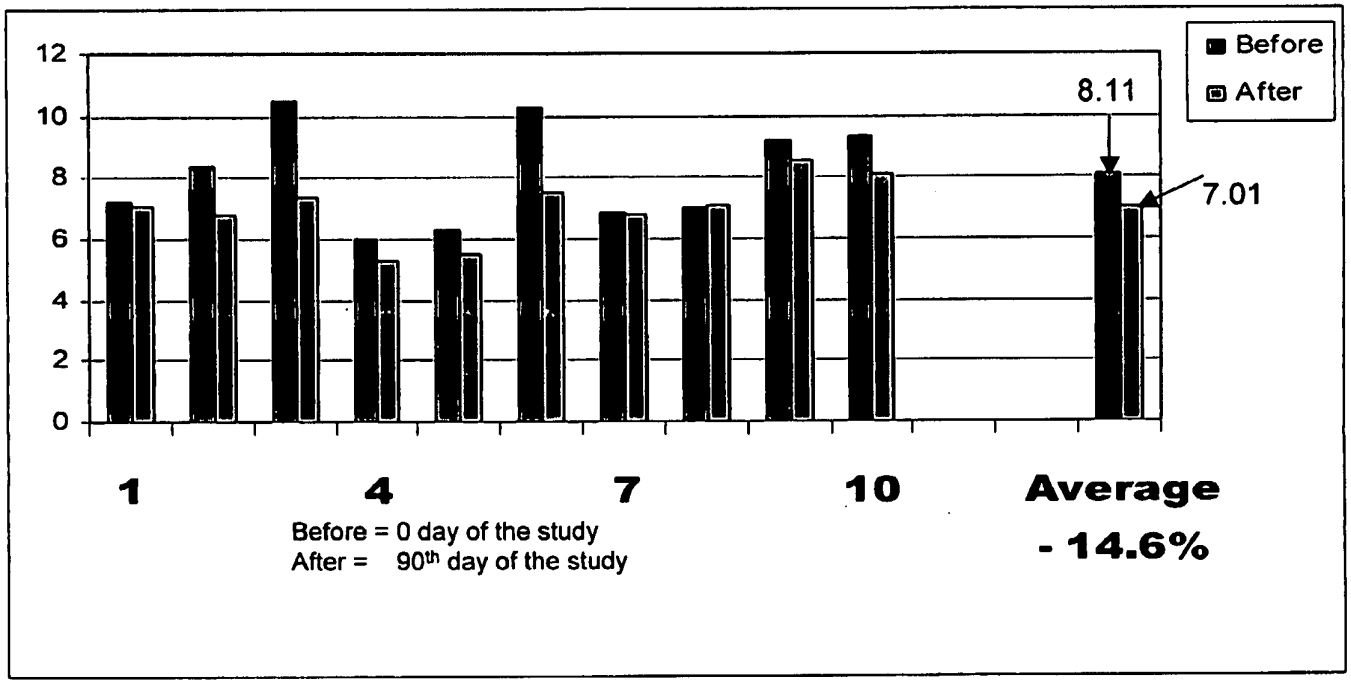
PILOT CLINICAL STUDY ON "PE1/PE2"

Glucosylated Hemoglobin Levels



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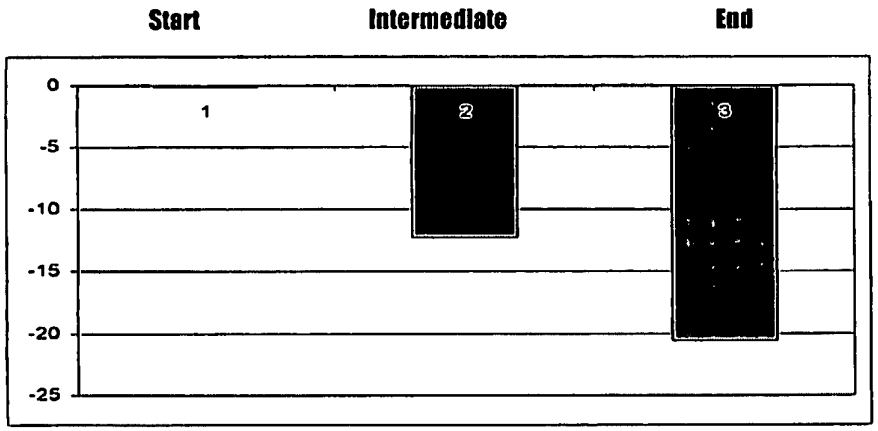
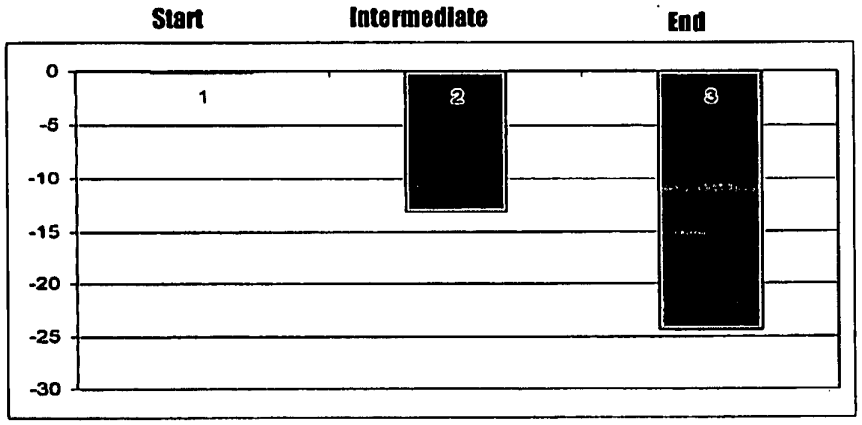
PILOT CLINICAL STUDY ON "PE1/PE2"

Levels of Fasting Glucose



Levels of Postprandial Glucose



Start = Relative Values at the beginning of the study (arbitrarily assigned 0 value)

Intermediate = Average value after 45th day of the study

End = Average value after 90th day of the study

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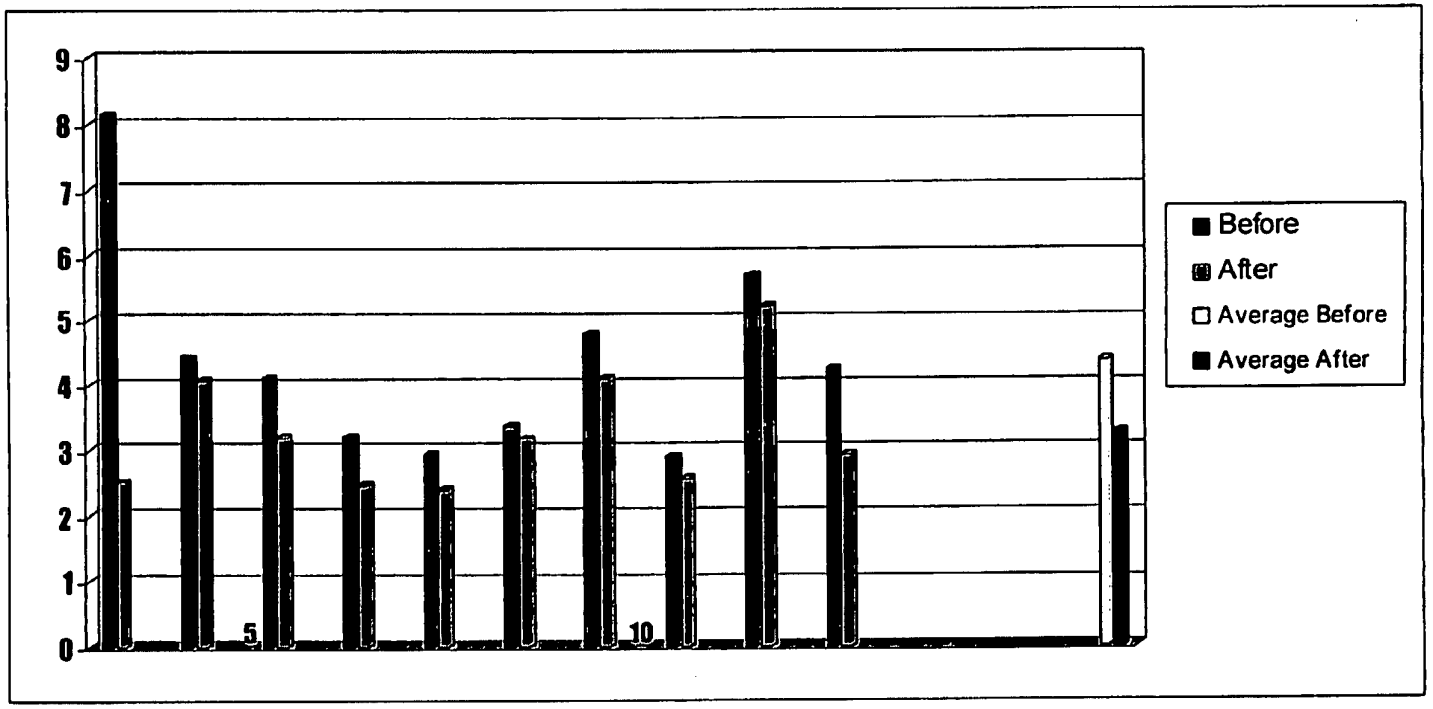
PILOT CLINICAL STUDY ON "PE1/PE2"

LDL/HDL Ratio



Before = 0 day of the study

After = 90th day of the study

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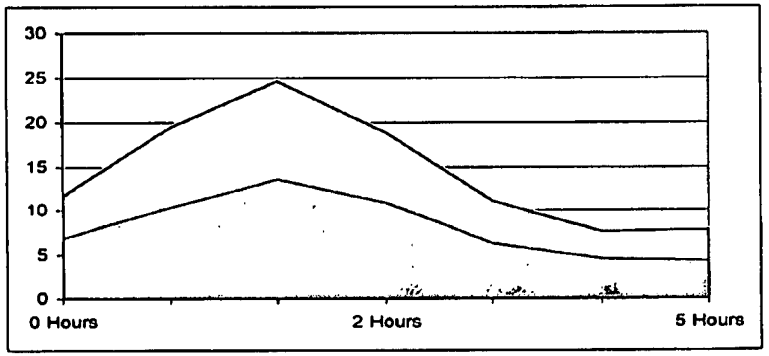
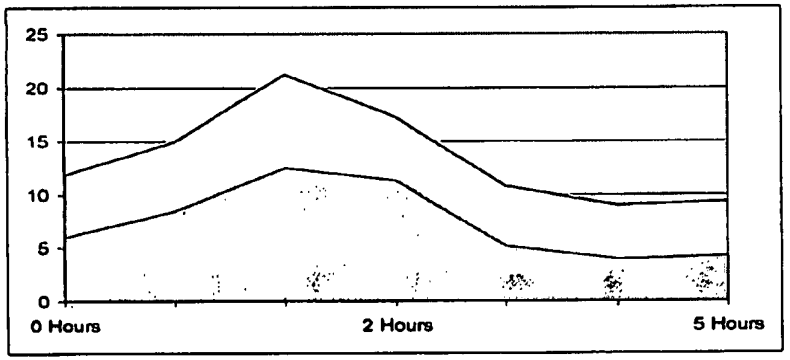
PILOT CLINICAL STUDY ON "PE1/PE2"

Oral Glucose Tolerance Test (OGTT)
on 2 Representative Patients

Patient 1



Patient 2



- Glucose Tolerance of Patient at the 0 day of the study
- Glucose Tolerance of the same patient after taking PE1/PE2 for 90 days

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PE1/PE2 extract showed hypoglycemic potency

Summary of Pilot Human Study

- Extract reduced fasted and postprandial glucose level in type 2 diabetic volunteers.
- Extract reduced HDL/LDL ratio and blood level of glycosylated hemogobin.
- Extract improved OGT and stimulated glucose transport to muscle cells.

Conclusions Drawn

- Extract contained some active principles that could be identified and developed.
- Active compounds might not be toxic since barley-based foods have been commonly used in the human diet for millennia.

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Characterization of the Active Principles from PE1/PE2

Discovery Phase Experiments

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IDENTIFICATION OF ACTIVE COMPOUNDS IN "PE1/PE2" EXTRACTS

- Selective and Specific Buffers and Solvent Mixtures, at different temperatures and contact times, were used for extraction of the active compounds.
- Membrane Filtration (Cut-off MW 1,000) was used.
- Preparative HPLC (C-18 column) was used to isolate individual compounds.
- H-1 and C-13 NMR Spectra and Mass Spectra were used for identification and structural determination purposes.
- Identified compounds subsequently individually screened for bioactivity.

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FEATURES of ACTIVE MOLECULES IDENTIFIED IN "PE1/PE2" EXTRACTS

- Identified compounds have MW below 1000.
- Certain of the identified compounds have been previously described in literature.
- Some of our compounds have pronounced biological activity unrelated to the scope of our research.
- Some of our compounds have novel structures.
- None of our compounds have been previously described for our suggested applications.
- Synthesis of all active molecules is relatively simple and does not require more than 3-5 steps.
- MitoChroma Research has identified synthesis routes for all active compounds.
- Identified compounds are stable in water solution.

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THE STRUCTURES OF NATURALLY OCCURRING AND SYNTHETIC COMPOUNDS THAT MODULATE GLUCOSE METABOLISM - EXAMPLES

Patented MitoChroma Discovery

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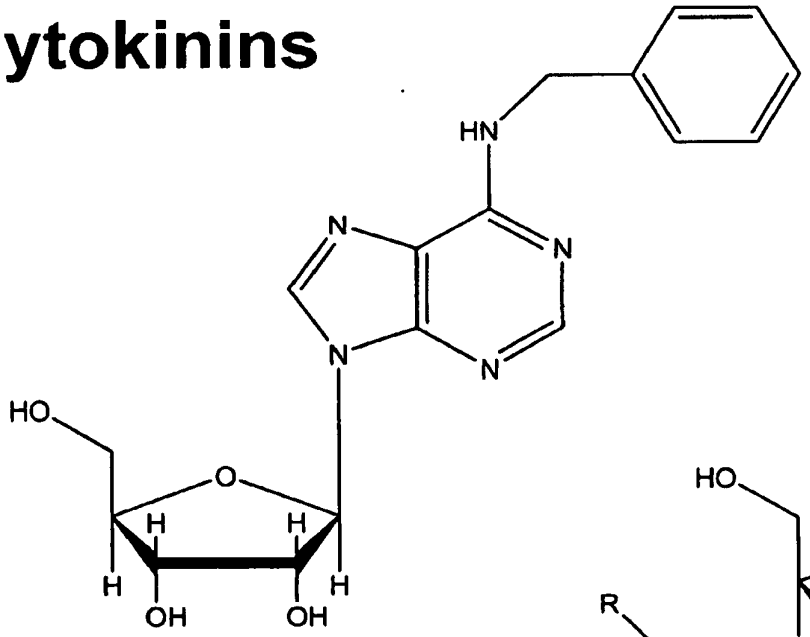
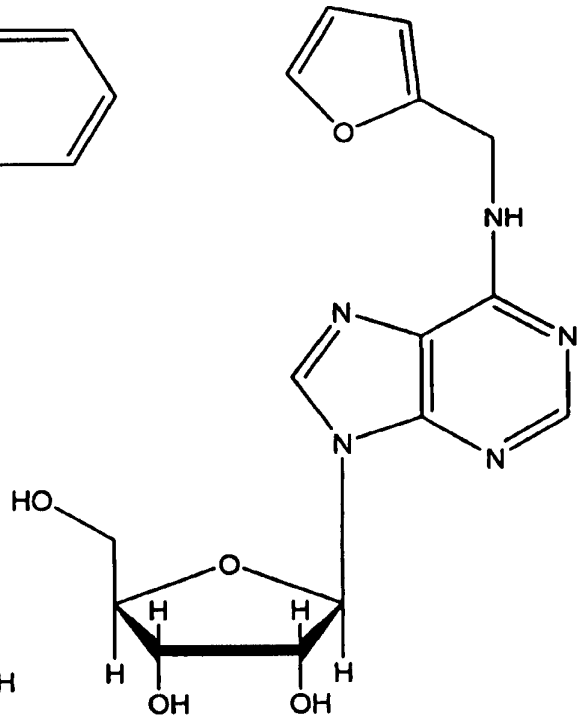
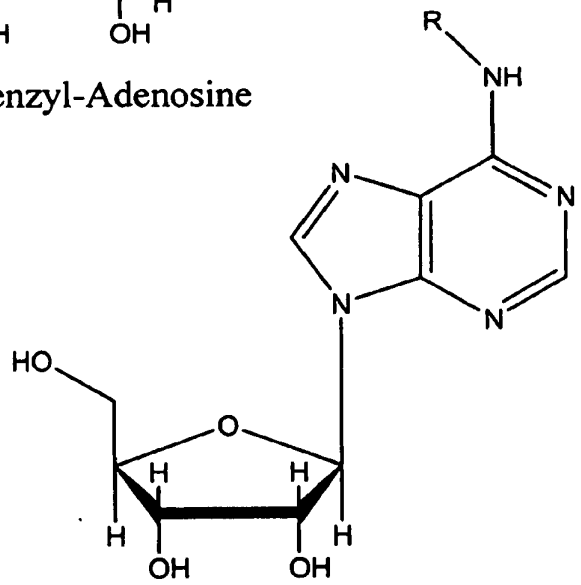
Synthetic Cytokinins



N6-Benzyl-Adenosine



Kinetin



Many other N6-Substituted 6-amino-purines

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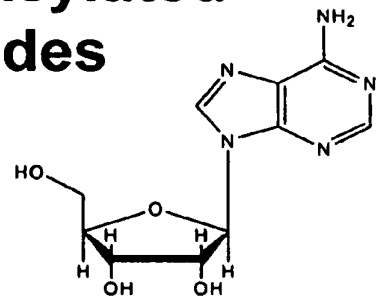
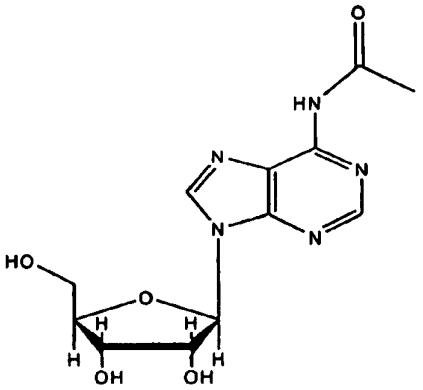
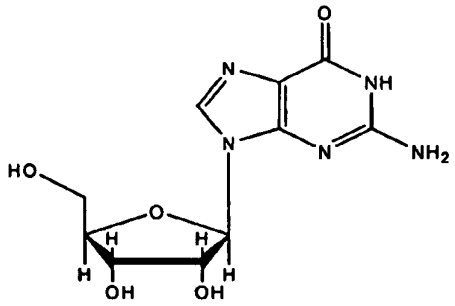
Synthetic N-Acylated Nucleosides



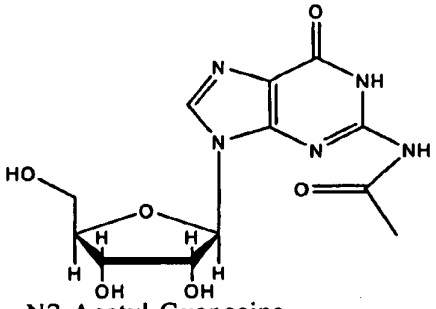
Adenosine



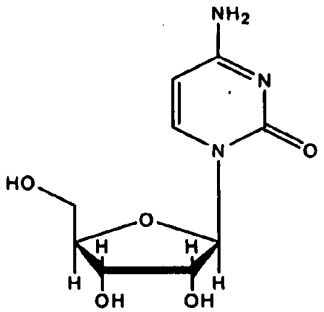
N6-Acetyl-Adenosine



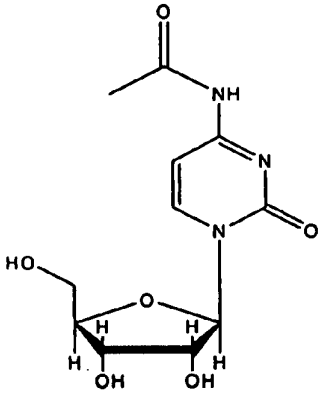
Guanosine



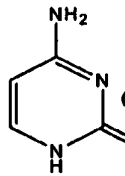
N2-Acetyl-Guanosine



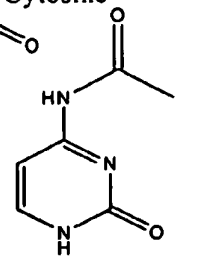
Cytidine



N4-Ac-Cytidine



Cytosine



N4-Acetyl-Cytosine

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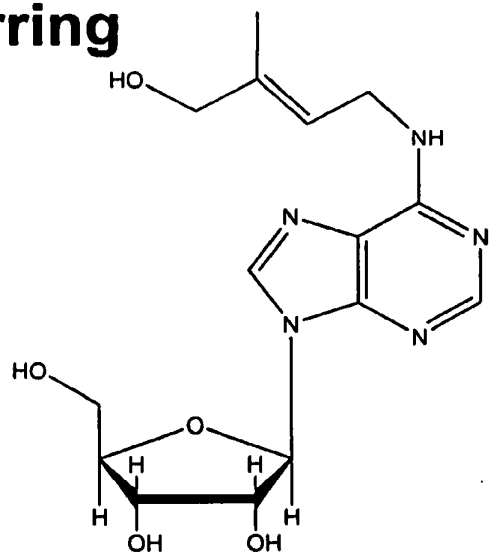
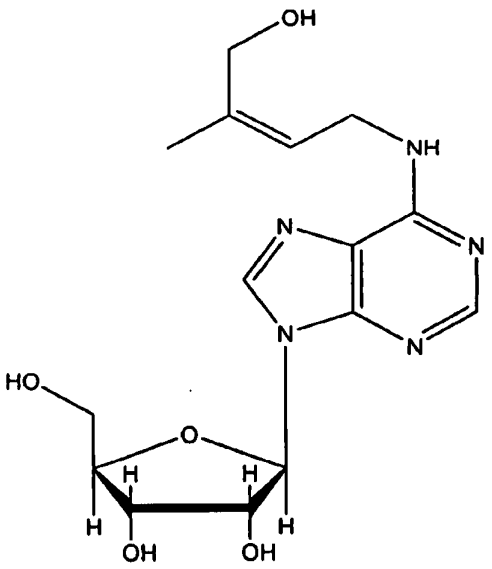
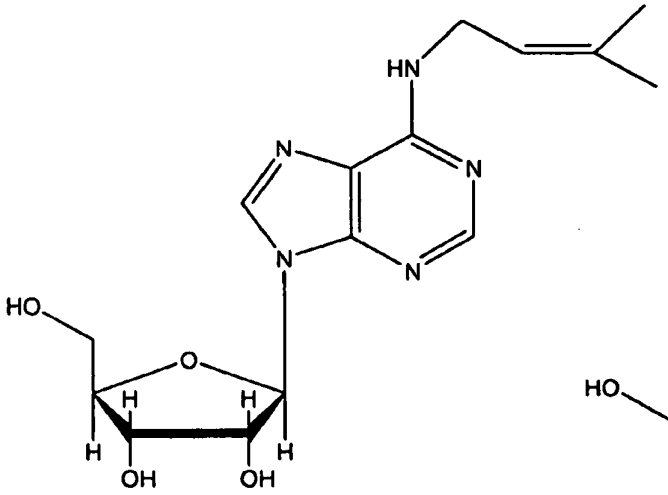
Naturally Occurring Cytokinins



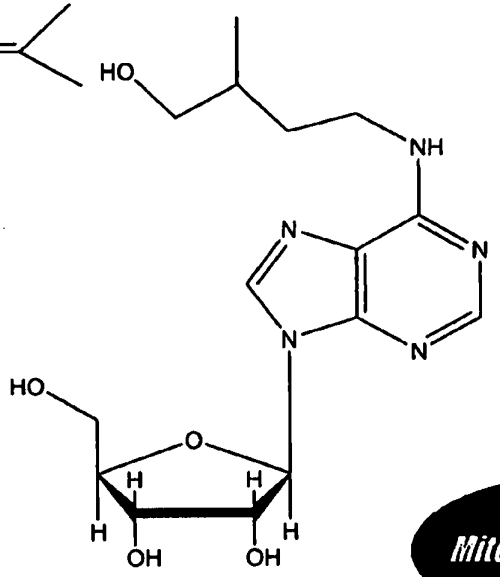
Trans-Zeatin



Cis-Zeatin



N6-Isopentenyl-Adenosine



Dihydro-Zeatin

MitoChroma Research

In Vitro Experiments On Our Individual Chemical Entities

Activity of AMPK and AKT in muscle cells

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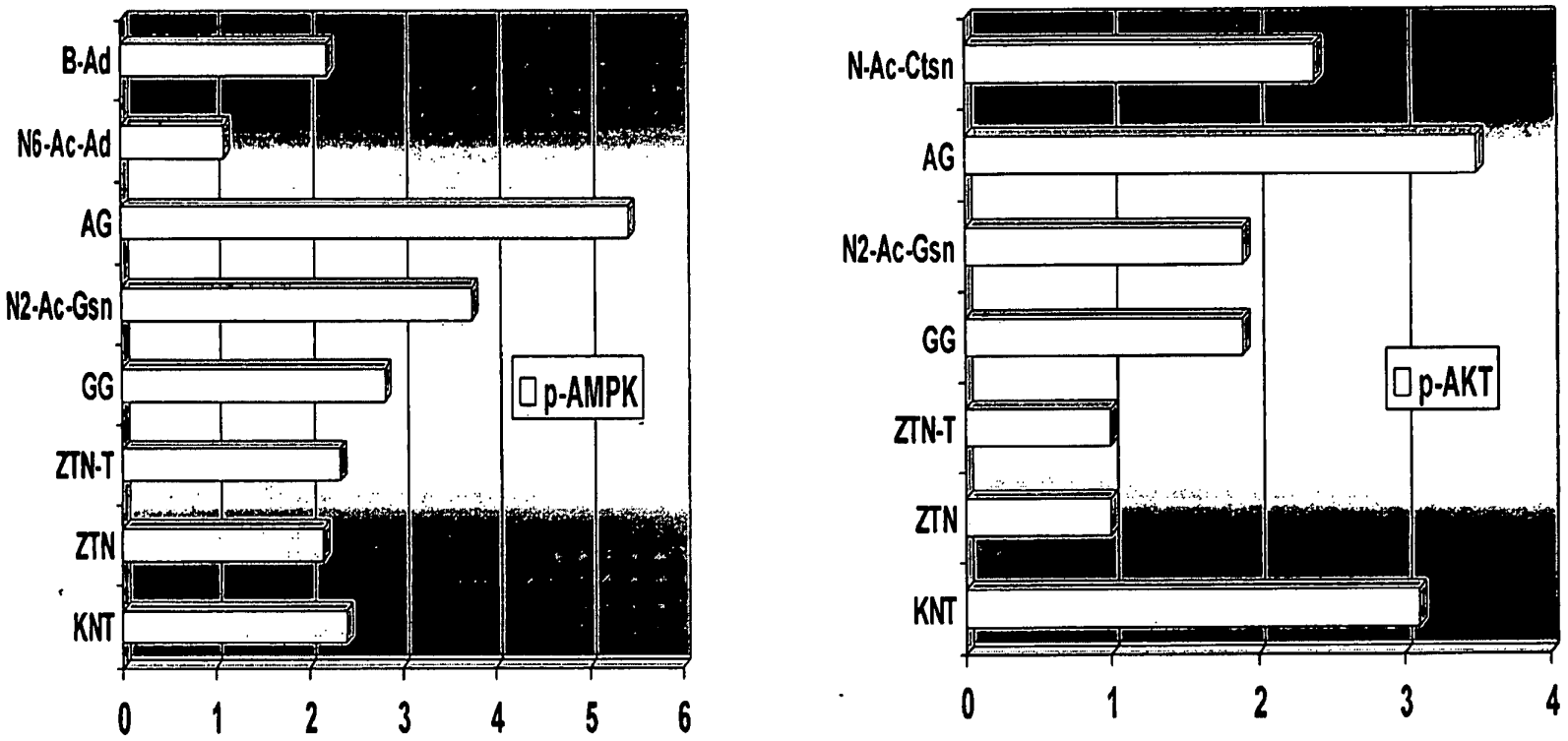
Level of p-AMPK and p-AKT in C2C12 muscle cells after treatment

Preliminary screening *In Vitro*



C2C12 cells were treated for 30 minutes at concentration 0.3-1uM. The level of p-AMPK and p-AKT was measured using antibodies against AMPK (Thr172) and AKT(Ser473).

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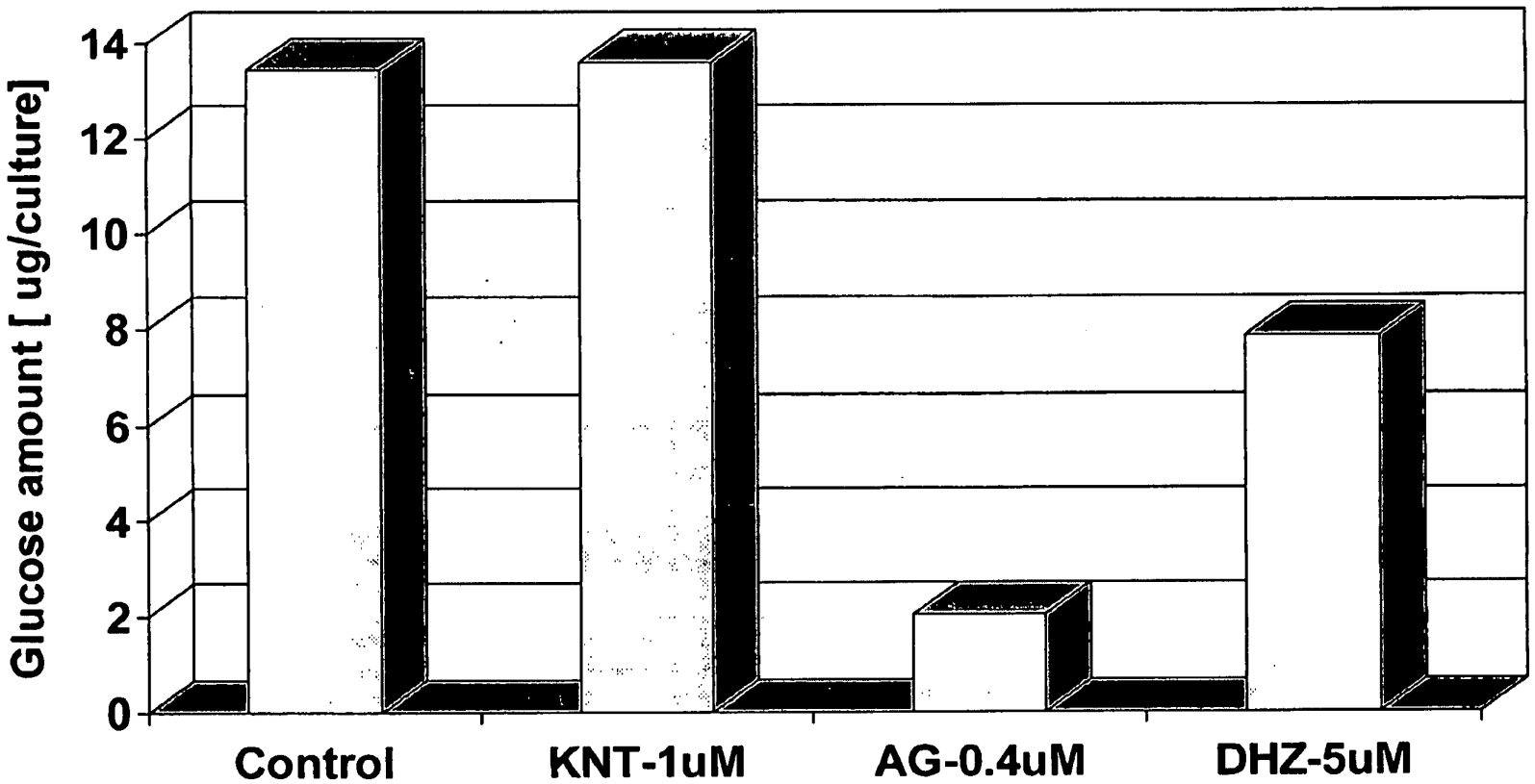
Effect of KNT, AG and DHZ on glucose output in HepG2 cells *in vitro* following 3 hrs treatment.



Tested compounds were not toxic under experimental conditions at concentrations up to 1mM as measured by MTT assay (EC50 is higher than 1mM).

More MT compounds are currently being tested under the same conditions. Time course and DRF are followed.

MitoChroma Research

Ex Vivo Experiments

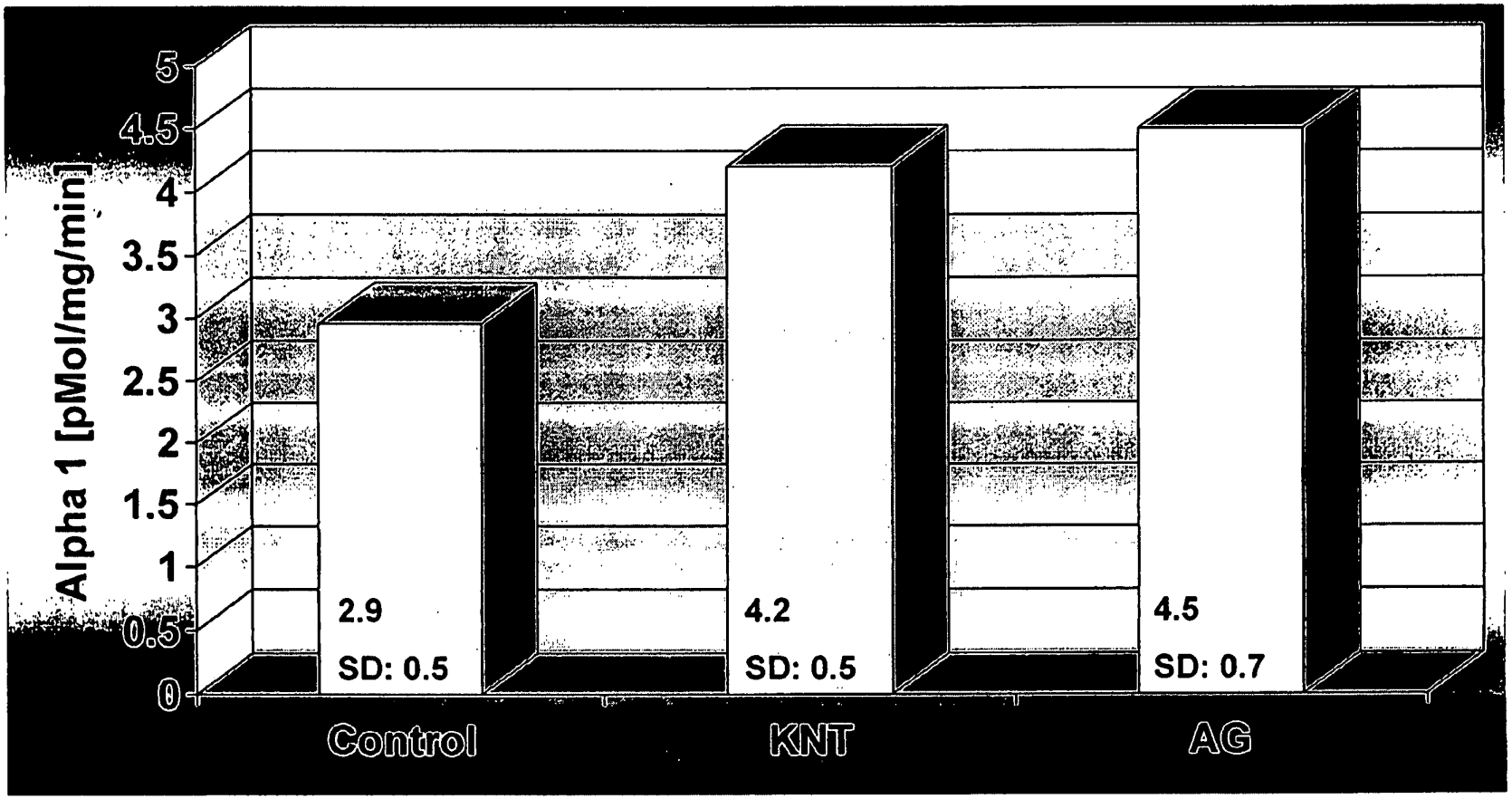
MitoChroma Research:

- **Dusan Miljkovic**
- **Jovan Hranisavljevic**
- **Zbigniew Pietrzkowski**

in cooperation with Joslin Diabetes Center:

- **Laurie Goodyear**
  - **Michael Hirshman**
  - **Nobuharu Fujii**

KNT and AG stimulate activity of AMPK alpha2 in Epitrochlearis muscles *ex vivo*



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AG but not KNT stimulates activity of AMPK alpha1 in Epitrochlearis muscles *ex vivo*

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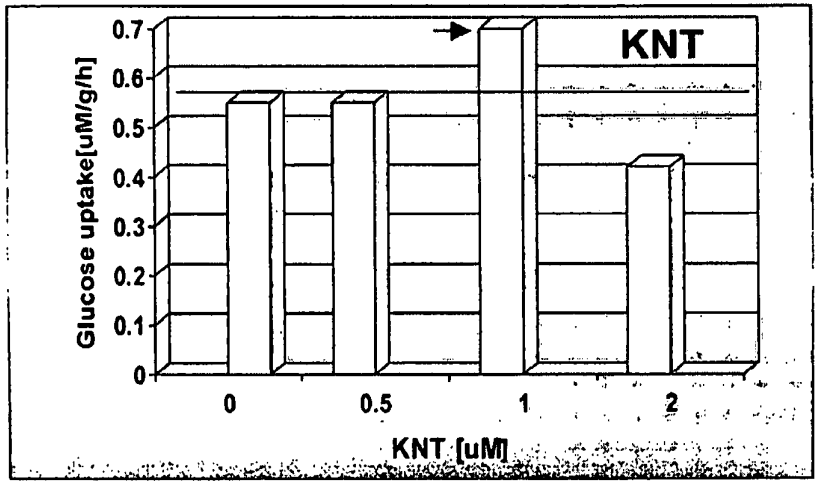
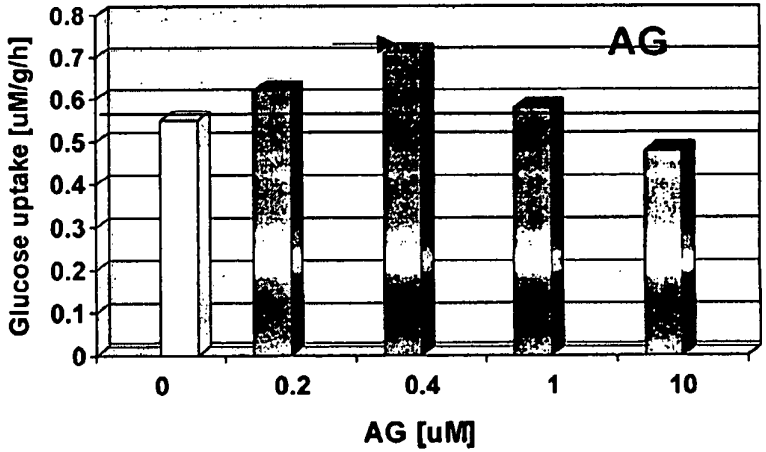
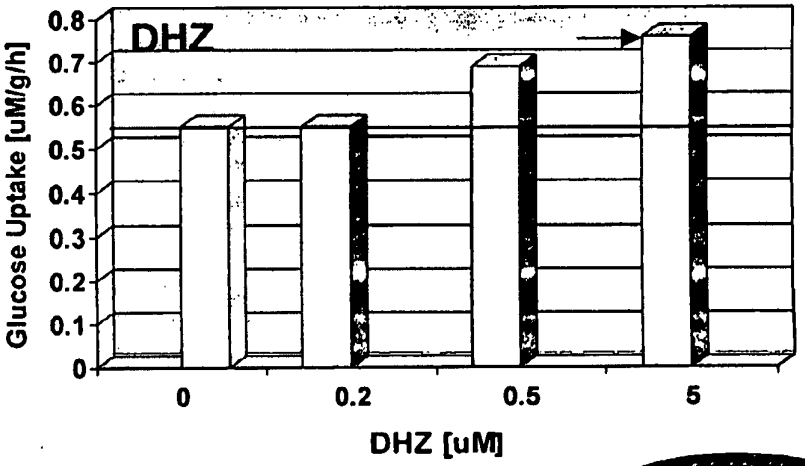
KNT, DHZ and AG stimulate glucose transport in rat Epitrochlearis muscles *ex vivo*







- 2-Deoxyglucose Uptake in rat Epitrochlearis Muscle
- 1 hr incubation at 37C, 10 min transport at 30C
- Arrows indicate significant stimulations

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Available Safety Data on Selected Compounds Within Our Class

- **Due to the commercial non-medical use of some of our compounds, there is a public body of mammalian toxicity data for such compounds.**
- **As an example we are providing published data on N-Benzyl-Adenine that has been compiled by the U.S. Environmental Protection Agency (EPA).**
- **Some of our related candidate compounds might have favorable ADMET characteristics.**

Subchronic Toxicity

- One of our active compounds (N-Benzyl-Adenine) has been used in commercial non-medical applications (in agriculture as a Cytokinin) and has been examined in detail by EPA for toxicity.

- 90-Day animal studies have been performed.

- Groups of Beagle dogs were fed diets containing the equivalent to mean intakes in excess of 26 mg/kg/day.

- No difference in weight gain was noted in any group. There were no affects on hematocrit, hemoglobin, RBC counts or WBC counts. Organ weights were comparable. Microscopic examination did not show evidence of treatment-related findings.

Certain of our compounds are in Toxicity Categories III and IV for acute oral, dermal, eye irritation and dermal irritation.

Category I = very highly or highly toxic
Category II = moderately toxic
Category III = slightly toxic
Category IV = practically non-toxic]

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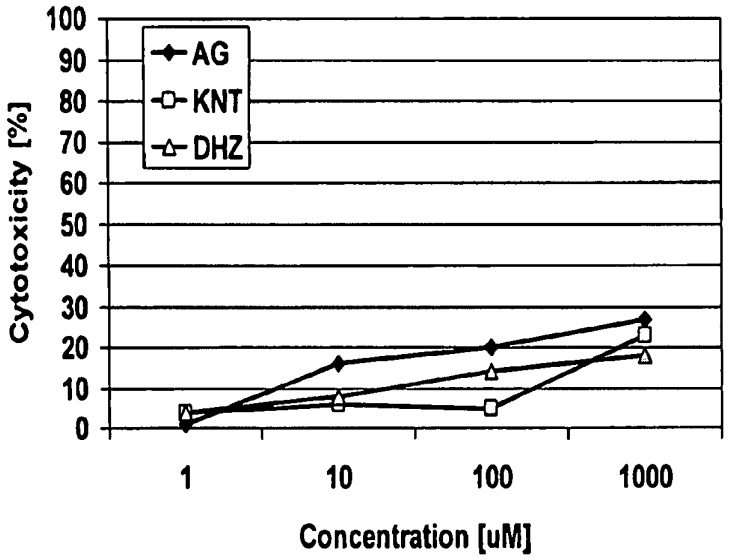
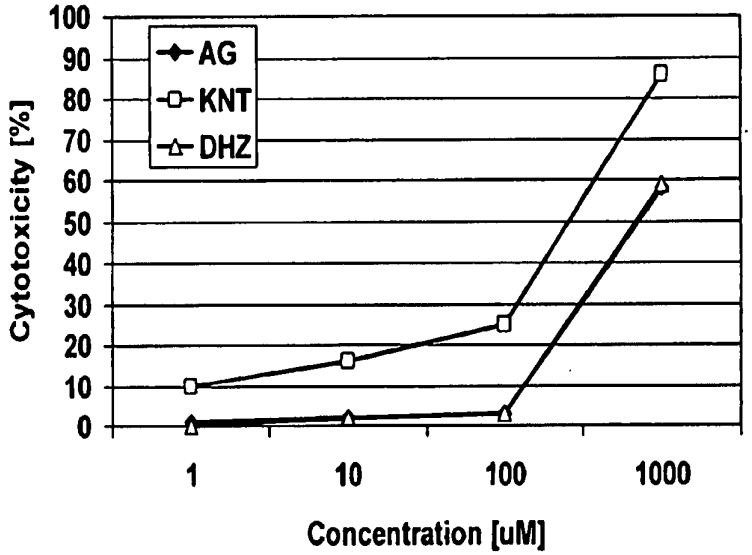
Cytotoxicity of KNT, DHZ and AG in culture of HepG2 cells and C2C12 muscle cells.



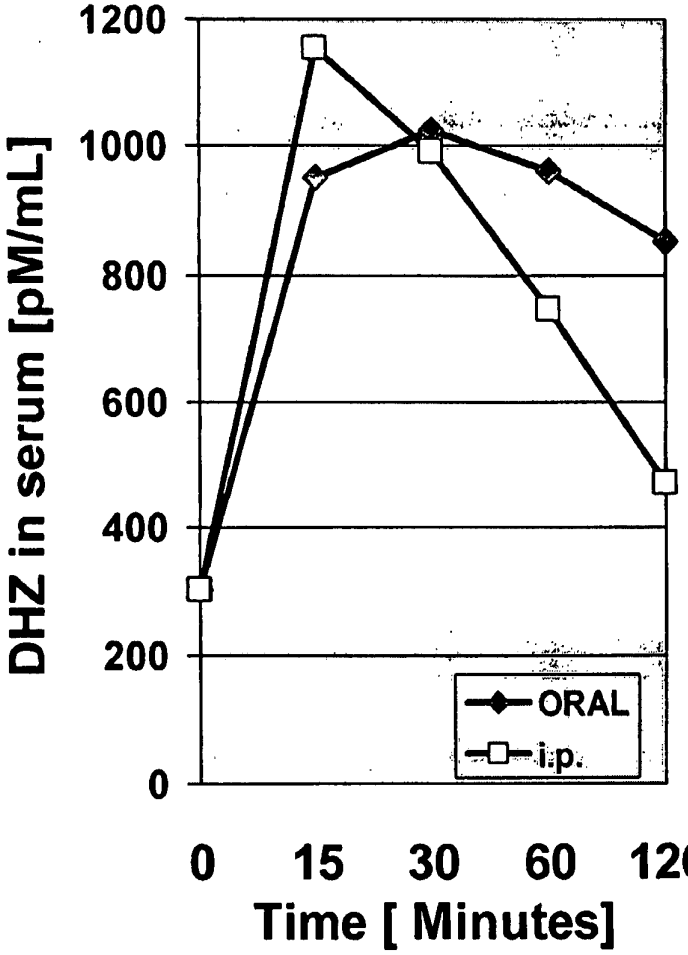


- Hepatic and muscle cells demonstrate different responses (toleration) to these three compounds.

MitoChroma Research

Bioavailability of DHZ in mice

Preliminary results



C57/Bl mice were treated with 100 ug/dose of DHZ for 0, 15, 30, 60 and 120 minutes following oral or i.p. administration.

Serum level of DHZ was measured using DHZ Elisa.

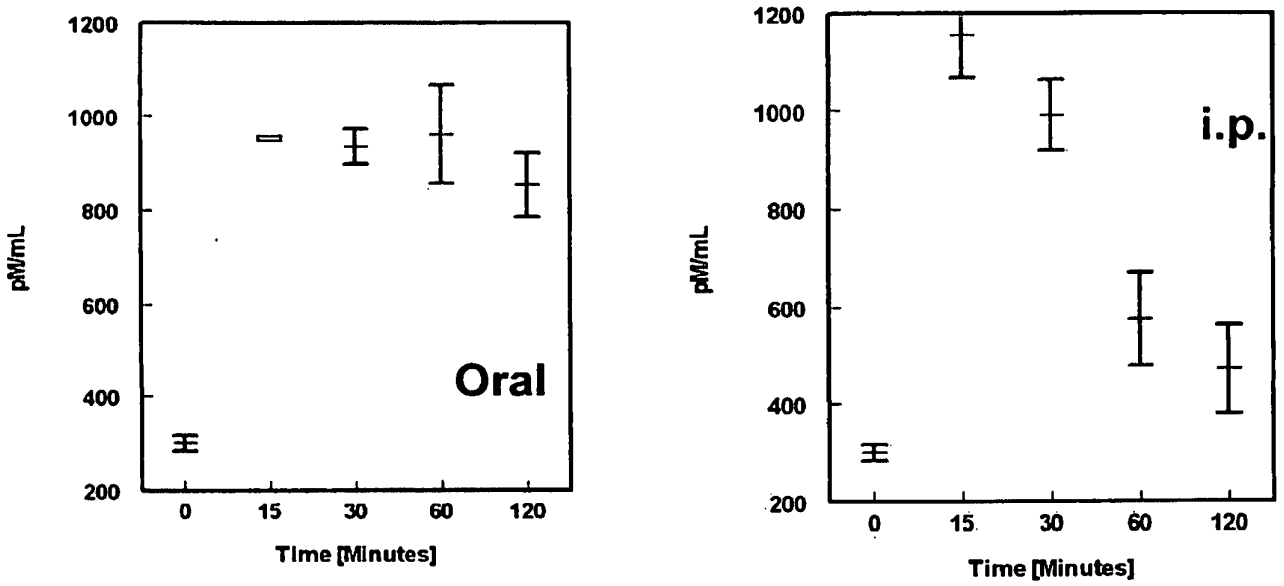
All animals survived the treatment and none exhibited signs of adverse effects.

Three animals were used per experimental point.

DHZ was very bioavailable following oral and i.p. administration

MitoChroma Research

Bioavailability of DHZ in mice



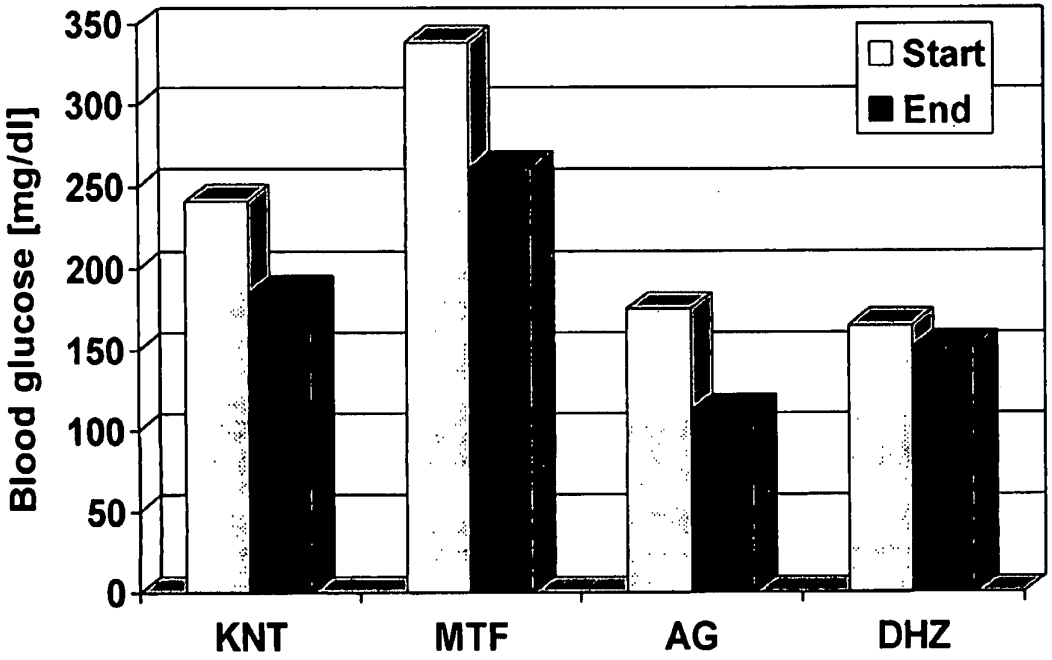
•C57/Bl mice were treated with DHZ (100ug/200ul) for 30, 60, 90 and 120 minutes. DHZ concentration in blood was measured using Elisa. Three animals per group were used in this first experiment.

•These results again show that DHZ is bioavailable.

MitoChroma Research

Acute hypoglycemic effect of KNT, MTF, DHZ and AG in fed db/db mice.

Preliminary Results



- *Treatment - 30' only*
- *Dose - 50ug/mouse*
- *Administration - i.p.*
- *Three mice per group*
- *Age - five weeks*
- *All animals were in good condition during this experiment*

- **Acute treatment reduced blood glucose significantly in animals treated with AG, MTF and KNT.**
- **DHZ induced only a 9% reduction in blood glucose levels under these experimental conditions.**

MitoChroma Research

Our compounds exhibit "Metformin-like" activity

Result-based comparison

Activity	Metformin	AG
Inhibition of Gluconeogenesis	Y	Y
Inhibition of PEPC	Y	P
Stimulation of glucose transport in		
Muscle	Y	Y
Adipocytes	Y	Y
AMPK activation	Y	Y

(P = possible)

MitoChroma Research

Pharmacological regulation of hepatic glucose production

Targets investigated by various laboratories

- Glucagon receptor
- Glycogen phosphorylase
- Glucocorticoid receptor
- 11-beta-hydroxysteroid dehydrogenase 1
- Fructose-1-6-bisphosphatase
- Carnitine palmitoyltransferase 1
- Glycogen synthetase -3,
- Glucose – 6 –phosphate T1 translocase
- A2B receptor
- Phosphoenolpyruvate carboxykinase

Ref: Curr Opin Investig Drugs. 2003, 4(4), 421-9, by Link JT

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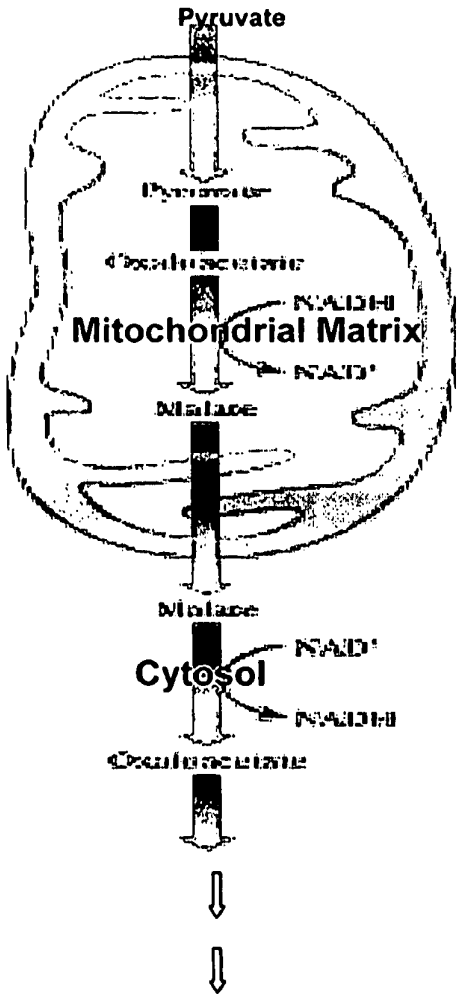
GLUCONEOGENESIS



GLUCONEOGENESIS

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Synthesis of (cytosolic) PEP from Pyruvate (in mitochondrial matrix)

3-step reaction:

pyruvate carboxylase

Pyruvate + CO_2 + ATP → oxaloacetate + ADP

Oxaloacetate + NADH → malate + NAD^+

malate dehydrogenase

Malate + NAD^+ → oxaloacetate + NADH

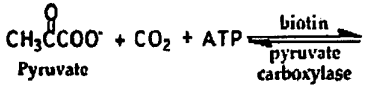
Phosphoenolpyruvate carboxylase

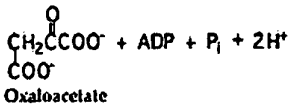
Oxaloacetate + GTP → PEP + GDP

Inhibitors of Phosphoenolpyruvate Carboxylase

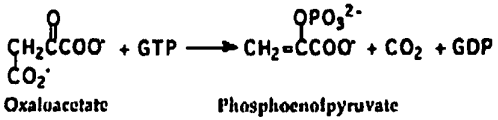
Step 1: carboxylation of pyruvate

- requires biotin
- pyruvate carboxylase is subject to allosteric control; it is activated by acetyl-CoA

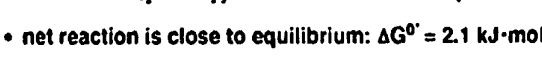




- decarboxylation of oxaloacetate is coupled with phosphorylation by GTP to give PEP



- the net reaction of carboxylation/decarboxylation is



- net reaction is close to equilibrium: $\Delta G^{0'} = 2.1$ kJ·mol^{-1}

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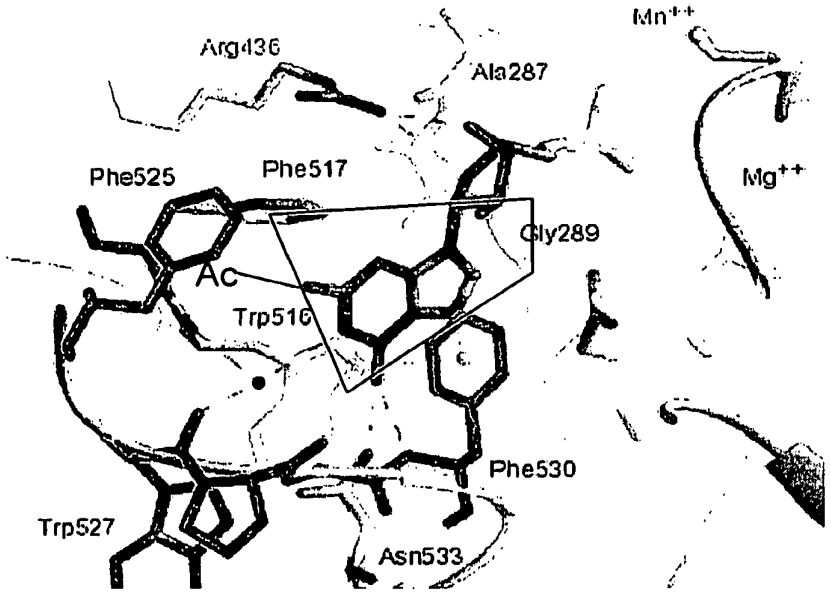
A POSSIBLE MECHANISM FOR AC-G ACTIVITY IN LIVER



Interactions between GTP and the active site of Phosphoenolpyruvate Carboxylase. N-2 Ac group and the red-framed area of GTP represent a hypothetical interaction of Ac-G that would inhibit the enzyme activity?

"GTP-binding site is unique to the GTP-dependent PEPCK family. The guanine binding pocket is an attractive target for inhibition by small molecules, given an opportunity for forming a number of hydrogen bonds in an otherwise HYDROPHOBIC environment shielded from water".

The above figure and the text fragment (in blue) to the right are taken from:

Crystal Structure of Human Cytosolic Phosphoenolpyruvate Carboxykinase Reveals a New GTP-binding Site

Pete Dunten*, Charles Belunis, Robert Crowther, Kurt Hollfelder, Ursula Kammlott, Wayne Levin, Hanspeter Michel, Gwendolyn B. Ramsey, Amy Swain, David Weber and Stanley J. Wertheimer

Roche Research Center
Hoffmann-La Roche Inc.
Nutley NJ 07110, USA

N-2-Ac group and the red frame were inserted by us as an illustration of our hypothesis

J. Mol. Biol. (2002) 316, 257-264

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Summary:

- Hypoglycemic extracts have been prepared from a variety of edible plant sources
  - Stimulate up to four-fold increase in fermentation rates and glucose uptake in yeast
  - Exhibit synergistic activity in combination on yeast fermentation rate
  - Stimulate glucose uptake in rat adipocytes and L6 muscle cells *in vitro*
  - Reduce blood glucose by nearly 55% in Streptozocin rats; significantly reduce liver enzymes and augment weight gains
- Small molecules have been isolated from edible plant extracts that exhibit the following properties:
  - Stimulate glucose uptake in EPI muscles *ex vivo* up to 45%
  - Increase AMPK activity in EPI muscles *ex vivo* up to 40%. MT1 stimulates both alpha 1 and alpha 2, however, MT7 preferably stimulates alpha 1 AMPK
  - Manifests activity at concentrations of 0.4-5uM.
- Our compounds are currently being investigated *in vitro* for inhibition of gluconeogenesis, and *in vivo* for hypoglycemic activity in diabetic animals.
  - According to preliminary results, some of our compounds show potent inhibitory effect on gluconeogenesis *in vitro*.
- Studies on preliminary toxicology (acute and long term), administration and metabolism are currently in preparation.

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MitoChroma Research Compounds: Near Future

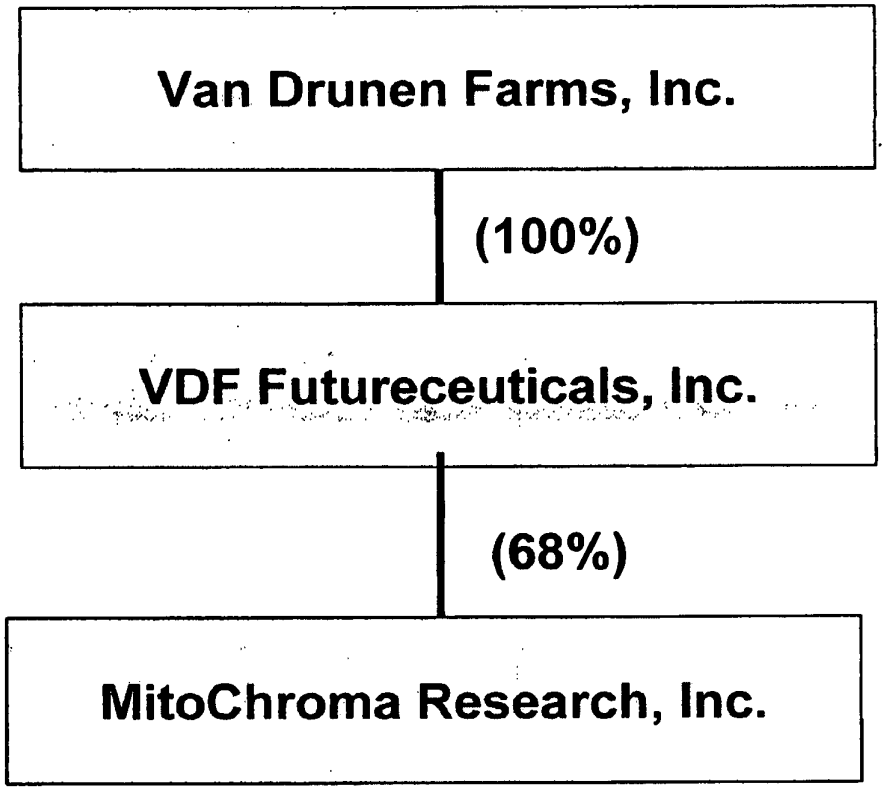
- Development of Metformin-like hypoglycemic medicines based on our compounds.
- Continued prosecution of our patent applications
- Collaboration with pharmaceutical partner.

MitoChroma Research

MitoChroma's Intellectual Property

- MitoChroma's patent portfolio currently comprises six patent applications initially filed either as U.S. patent applications or under the Patent Cooperation Treaty.
- We have licensed certain rights under our intellectual property to our parent for application in the field of nutritional supplements.
- The claims in our applications include compositions of matter, manufacturing methods, and treatment methods.

MitoChroma's Corporate Structure



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MitoChroma's Management and Scientists

Jeff Van Drunen	President, Chairman
John Hunter	Vice President – Scientific and Business Development, Director
Dusan Miljkovic, Ph.D.	Vice President – Research & Development, Chief Scientific Officer, Director
Zbigniew Pietrzkowski, Ph.D.	Director of Biology
Jovan Hranisavljevic, Ph.D.	Scientific Advisor

MitoChroma Research

A NATURALLY OCCURRING COMPOUND THAT INCREASES GLUCOSE UPTAKE AND AMP-ACTIVATED PROTEIN KINASE ACTIVITY IN MUSCLE

D Miljkovic[1], MF Hirshman[2], Z Pietrzkowski[1], J Hranisavljevic[1], V Miljkovic[1], N Fujii[2], J Pomerleau[2], J Hunter[1], and LJ Goodyear[2]. [1]MitoChroma Research, Momence, USA, and [2]Joslin Diabetes Center and Harvard Medical School, Boston, USA

Based upon the known abilities of certain plant varieties to modulate blood glucose, the goal of this study was to isolate and identify the active substances and to investigate these compounds for stimulation of glucose uptake and AMPK activity. Plant materials were initially extracted with ethanol and various buffers. Numerous fractions (with m.w. below 1,000 Daltons) were separated by semi-preparative HPLC. Several active compounds were isolated and structures identified by M-spectrometry and NMR-spectroscopy. Fractions, as well as subsequent individual compounds, were initially screened for potential activation of glucose transport and AMPK *in vitro* using differentiated C2C12 muscle cells. As an example we report results with one compound, (working name "MTO"), as a representative of a broad class of compounds we are investigating. MTO increased both AMPK^{Thr172} phosphorylation and glucose uptake (Table; n=5-8/group; fold-increase over control).

Concentration [μM]	p-AMPK fold increase	Glucose uptake fold increase
0.3	1.6	3.0
1.0	2.4	3.3
3.0	2.6	2.2

We next determined the effects of MTO on 2-deoxyglucose uptake and AMPK in rat epitrochlearis muscles *ex vivo* (n=5-8/group). Isolated muscles were incubated with 1 μM for 0.5, 1 and 2 h and at concentrations ranging from 0.2-50 μM. MTO increased 2-deoxyglucose uptake at 1 and 2 h, but not at 30 min. Lower concentrations increased 2-deoxyglucose uptake, peaking at 1 μM (44% above basal) and decreasing back to baseline rates at higher concentrations. Incubation (1 μM, 1 h) increased AMPKα1 activity by 47%, and there was a trend to increase AMPKα2 activity (23%), although this did not reach statistical significance. AMPK^{Thr172} phosphorylation was increased by 57%. In conclusion, the low, systemically achievable, micro- and nanomolar concentrations of MTO that stimulated glucose uptake and AMPK activation suggests that this compound, and others from the class, merit further research and development for metabolic disease applications.

CONFIDENTIAL (Abstract from the Book of Abstracts, AMPK 2004, Australia)

MitoChroma Research

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/025026

International filing date: 03 August 2004 (03.08.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/562,496
Filing date: 14 April 2004 (14.04.2004)

Date of receipt at the International Bureau: 23 September 2004 (23.09.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

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